

U.S. Serial No. 09/811,768
Amendment under 37 CFR §1.312

IN THE SPECIFICATION:

Please amend the paragraph beginning at page 1, line 25 as follows:

United States Patent No. 5180597 ~~claimed~~ sets forth a process for hydrolyzed vegetable protein with enhanced flavor, which contains no detectable level of monochlorodihydroxypropanol, ~~is described~~. In the above reference, wheat gluten is hydrolyzed using Prozyme 6 (a fungal protease) at a temperature of 40-50°C, pH 6.5-7.0, with an enzyme concentration of 0.1-2.0% of substrate for a time period of 4h. The hydrolyzed protein is treated with gaseous HCl for deamidation before the addition of acid for inactivating the enzyme. The drawback in such hydrolysis is that it is likely to lead to racemisation of amino acids and the addition of acid increases the salt content in the product.

Please amend the paragraph beginning at page 2, line 21 as follows:

Reference may be made to Cipollo, K.L. and Wagner, T.J., (1987) European Patent No. 0148600 B 1, Ralston Purina Co., wherein the described process relates to the preparation of hydrolyzed protein from protein isolate after jet cooking or dynamic heating at 104°C for a few seconds and later cooled in a vacuum chamber before hydrolysis using bromelain. The protein was precipitated at its isoelectric point from an aqueous extract of the material before the hydrolysis. The drawback of the process is the starting material protein isolate, which is ~~more~~ expensive. The process is a multi-step process, energy intensive. The process further needs machines like the jet cooker and a vacuum chamber.

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Please amend the paragraph beginning at page 3, line 13 as follows:

Reference may be made to a Boyce, C. O. L. *et al.*, (1986), European Patent No. 0187048 A2, NOVO Industries A/S, wherein a process is described for the preparation of soy protein hydrolysate with 0.25 to 2.5% degree of hydrolysis (DH) using microbial rennet (*Mucor miehei*) and to be used as an egg white substitute. The enzyme used in this process is different and involves a low degree of hydrolysis of soy protein.

Please amend the paragraph beginning at page 3, line 18 as follows:

Reference may be made to Olsen, H. A. S. (1981), United Kingdom Patent No. 2053228A, wherein a process for the production of soy protein hydrolysate from partially defatted soy material by hydrolysis with proteolytic enzyme. The drawback of the process is that due to partial defatting soy flour, left-over oil comes in contact with protein phase, which could lead to off-flavors.

Please amend the paragraph beginning at page 3, line 23 as follows:

Reference may be made to Olsen, H. S. (1981) United States Patent No. 4324805, wherein method described for producing soy protein hydrolysate and oil from partially defatted soy material by hydrolysis with proteolytic enzyme. The soy flour is partially defatted by water washing at pH 3.5-4.5 and later hydrolyzed using water and a base to increase the pH. The degree of hydrolysis (DH) is in the range of 8-12%. Oil is recovered from the wash water. Alcalase is the

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enzyme used. The drawback of the process is that it is a multi-step process and, due to partial defatting of soy flour, left-over oil comes in contact with protein phase which could lead to off-flavors. Enzyme inactivation is done by addition of acid, which is likely to lead to increased salt content in the product.

Please amend the paragraph beginning at page 4, line 13 as follows:

Reference may be made to Gunther, R.C. (1972) Canadian Patent No. 905742, wherein soy protein hydrolysate is modified with pepsin to yield a product, which, in the presence of water and sugar, whips at a rapid rate to produce aerated products of low density.

Please amend the paragraph beginning at page 4, line 21 as follows:

Reference may be made to a published paper entitled "Industrial pProduction and aApplication of eSoluble eEnzymatic hHydrolysate of eSoy pProtein", by Olsen, H.S, Adler, Nissen, J. (1979), Process Biochemistry, 14(7), 6,8, 10-11, wherein a method for the preparation of soy protein hydrolysate from soy flakes washed at pH 4.5 followed by hydrolysis using alcalase is described. The solubility of the substrate is low at the acidic pH which is likely to result in low yields. The enzyme used is different from the enzyme used in the present invention.

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Please amend the paragraph beginning at page 5, line 16 as follows:

Accordingly, the present invention provides a process for the preparation of protein hydrolysate from soy flour, which comprises preparing an aqueous slurry of defatted soy flour having 6-12% w/v of solid content, hydrolyzing the ~~said~~ slurry using fungal protease at pH 7-8 and a temperature of $43 \pm 5^{\circ}\text{C}$ to get 20-40% DH, further hydrolyzing using papain at a temperature of $53 \pm 5^{\circ}\text{C}$ under stirring till completion of hydrolysis to 30 – 45% DH, inactivating the residual enzyme in a known manner, and separating the solids and drying the clarified supernatant thus obtained to get protein hydrolysate.

Please amend the paragraph beginning at page 6, line 2 as follows:

Accordingly, the present invention provides a process for the preparation of protein hydrolysate from soy flour using fungal protease, ~~said the~~ process comprising: preparing an aqueous slurry of defatted soy flour having 6-12% w/v of solid content; hydrolyzing the ~~said~~-slurry using fungal protease at pH 7-8 and a temperature of $43 \pm 5^{\circ}\text{C}$ to get 20-40% degree of hydrolysis (DH); further hydrolyzing using papain at a temperature of $53 \pm 5^{\circ}\text{C}$ under stirring till 30 – 45% DH is obtained, inactivating residual enzyme in a known manner, and separating the solids and drying the clarified supernatant thus obtained to get protein hydrolysate.

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Please amend the paragraph beginning at page 6, line 14 as follows:

In still another embodiment of the present invention, the *Aspergillus* is selected from the group comprising ~~of~~ *A. flavus*, *A. japonicus*, *A. niger* and *A. awamori*.

Please amend the paragraph beginning at page 7, line 9 as follows:

In one ~~another~~ other of the present invention, a high yield of protein hydrolysate with 35 to 45% degree of hydrolysis is obtained from the raw material taken.

Please amend the paragraph beginning at page 7, line 11 as follows:

In an embodiment of the present invention, the soy protein hydrolysate obtained has creamy color and a yield of 60-67.0% (on protein basis).

Please amend the paragraph beginning at page 7, line 20 as follows:

In one more embodiment of the present invention, the amino acid composition of the protein hydrolysate is similar to the amino acid makeup of the starting material.

Please amend the paragraph beginning at page 9, line 21 as follows:

Defatted soy bean flour was dispersed in water with a suitable solvent to solute ratio and the pH of the dispersion was adjusted using 6N sodium hydroxide or 6N hydrochloric acid. This was ~~kept stirring~~ stirred for a few

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minutes with a mechanical stirrer and then the temperature was raised to 40 to 45°C. At this stage 0.4 to 0.5% of fungal enzyme on the basis of soy flour was added and stirring continued for 2 hours. At the end of stipulated time, the temperature of the slurry was raised ~~to~~ 50 to 55°C. To this, 0.4 to 0.5% of papain on the basis of soy flour was added and stirring continued for 1 to 2 hours. At the end of the above time interval, the temperature of the slurry was raised ~~to~~ 90 to 95°C for 5 to 10 minutes. The slurry was cooled to room temperature and the insoluble carbohydrate-rich fraction was removed by centrifugation. The clarified protein hydrolysate was spray dried to obtain protein hydrolysate.

Please amend the paragraph beginning at page 10, line 6 as follows:

Twenty-five grams of defatted soy flour is were dispersed in 250 ml of water and the pH of the dispersion was adjusted to 7.2 ~~by~~ using 6N sodium hydroxide solution. It was ~~kept stirring~~ stirred for 20 min with a mechanical stirrer and the temperature was raised to 40°C by heating. At this stage, 125 mg of fungal protease was added and stirring continued for 2 hours. At the end of 2 hours, the temperature was raised to 50°C by heating and the second enzyme, papain (125 mg), was added and ~~kept stirring~~ stirred for 1 hour. After the hydrolysis, the resultant solution was boiled for 10 min for enzyme inactivation. The slurry was centrifuged using a basket centrifuge. The clear solution was lyophilised. The yield was 65% on protein basis and the degree of hydrolysis by TNBS method was found to be 43%.

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Please amend the paragraph beginning at page 10, line 16 as follows:

Fifty grams of soy flour ~~is~~ were dispersed in 500 ml of water and the pH of the dispersion was adjusted to 7.3. It was ~~kept stirring~~ stirred for 20 min with a mechanical stirrer and the temperature was raised to 43°C. At this stage of 250 mg of fungal protease ~~is~~ was added and stirring was continued for 1.5 hours. At the end of 2 hours the temperature was raised to 53°C and the second enzyme papain (250 mg) was added and ~~kept stirring~~ stirred for 1 hours. After the hydrolysis the hydrolysate was boiled for 15 min. for enzyme inactivation and was centrifuged. The clear solution was lyophilised. The yield was 68.0% on protein basis and degree of hydrolysis by TNBS method was 39%.

Please amend the paragraph beginning at page 10, line 25 as follows:

One hundred grams of defatted soybean flour ~~is~~ was dispersed in 1 L of water and the pH of the dispersion was adjusted to 7.6. It was ~~kept stirring~~ stirred for 20 min with a mechanical stirrer and then temperature was raised to 45°C. At this stage 500 mg of fungal protease ~~is~~ was added and stirring continued for 2 hours. At the end of 2 hours the temperature was raised to 55°C and the second enzyme papain 500 mg was added and ~~kept stirring~~ was stirred for 1.5 hours. After the hydrolysis the hydrolysate was boiled for 10 minutes for enzyme inactivation and was centrifuged. The clear solution was spray dried. The yield was 70% on protein basis and degree of hydrolysis by TNBS method was 38%.

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Please amend the paragraph beginning at page 11, line 7 as follows:

One kg of soy flour ~~is~~ was dispersed in 10 L of water and the pH of the dispersion was adjusted to 7.6. It was kept stirring for 15 minutes with a mechanical stirrer and then the temperature was raised to 45°C. At this stage, 5 g of fungal protease ~~is~~ was added and stirring continued for 2 hours. At the end of 2 hours, the slurry temperature was raised to 55°C and the second enzyme papain (5g) was added and ~~kept stirring~~ stirred for 1.5 hours. After hydrolysis, the hydrolysate was boiled for 15 minutes for enzyme inactivation and was centrifuged in ~~the~~ a basket centrifuge. The clear solution was spray dried. The degree of hydrolysis was found to be 38 % and the yield was 70% on a protein basis.

Please amend the paragraph beginning at page 11, line 15 as follows:

The particle size of the soy flour, ratio of enzyme to substrate, temperature, pH and time interval controls the end of enzymatic hydrolysis resulting ~~into minimizing~~ in minimal bitterness of the hydrolysate.

Please amend the paragraph beginning at page 12, line 4 as follows:

1. By using this process, the product attains a property of ~~becoming~~ being a good additive without imparting any undesirable odd flavour ~~for~~ to the finished product.